Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Bio-conjugated carbon dots for bimodal detection of prostate cancer biomarker via sandwich fluorescence and electrochemical immunoassay

Jyoti Korram[a,1], Amarnath Chellachamy Anbalagan[a, 1], Anannya Banerjee[b] and Shilpa N. Sawant [a, c, *]

[a] *Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India*

[b] *Fuel Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India*

[c] *Homi Bhabha National Institute, Anushaktinagar, Mumbai 400094, India.*

[1] *Equal contribution*

Correspondence: Telephone: +91 22 25593869, Email: stawde@barc.gov.in (Dr. Shilpa N. Sawant)

Materials

Aniline, ammonium persulfate, hydrochloric acid, polyethyleneimine, bovine serum albumin, hydroquinone, hydrogen peroxide and human serum were purchased from Sigma-Aldrich (Merck). Onions as precursor were collected from local market. Monoclonal PSA antibody ($^{PSA}Ab_{cap}$), HRP conjugated polyclonal PSA antibody ($^{PSA}Ab_{HRP}$) and PSA were procured from MyBioSource, USA. Screen Printed Electrode (SPE) purchase from IPGI Chennai India. All other reagents were of analytical grade and were used without further purification. Phosphate buffer solution (pH 7.4) was made by mixing the solutions of dibasic and monobasic sodium phosphate and used as the electrolyte.

Characterization

The absorption and fluorescence spectra of CDs were acquired with a UV-Visible spectrophotometer (JASCO-UV-650) and spectrofluorometer (JASCO FP-8500), respectively. The functional groups of CDs were analysed using a Fourier Transform Infrared (FTIR) spectrometer (Bruker, ALPHA II Compact). Elemental analysis was investigated using Energy dispersive X-ray spectroscopy (EDX), JOEL, Japan. The morphology and particle size of C-dots were examined by transmission electron microscopy (TEM, Thermo Scientific 300 G3 microscope) with an accelerating voltage of 300 KV. Electrochemical studies like cyclic voltammetry and chronoamperometry (CA) were perform in Metrohm Autolab electrochemical work station. Electrochemical biosensor electrodes were designed by drop-casting aqueous PANI NPs onto a working electrode of SPE followed by drying at room temperature for 30 min. Concentration of PSA in human blood serum was also determined by conventional Chemiluminescence Immunoassay (CLIA) technique.

FT-IR of OCDs

The surface functional groups of OCDs were recognized by FTIR spectroscopy. As shown in Fig. 1c, the broad peaks from 3200 to 3400 cm^{-1} was attributed to stretching vibrations of O-H and N-H [1] The S-H stretching vibration of a thiol was observed between 2550–2600 cm^{-1} region. The absorption of the S-H bond is much less intense than that of the O-H bond because the S-H bond is less polar. In addition, the S-H absorption is not broad because S-H does not form intramolecular hydrogen bonds. The peaks in the region of 1575-1700 cm^{-1} represented stretching vibration of C-O-C, C=C, and bending vibration of COO^- [2]. In addition, the peaks at about 1300 cm^{-1} can be ascribed to the stretching vibration of C-S suggesting the existence of sulfur in the CDs [3]. Besides, the peaks in the range of 600-700 cm^{-1} can be ascribed to C-H and C-O groups. The FTIR spectra emphasized the incorporation of the amino, hydroxyl, carboxylic and sulfur containing groups on the surface of OCDs, conferring high polarity and water solubility of the OCDs [4]. The FTIR spectra of onion biomass, precursor of OCDs was also recorded. It displayed characteristic stretching vibration of C-S and a low intensity absorption due to the S-H bond suggesting the existence of sulfur in the onion biomass.

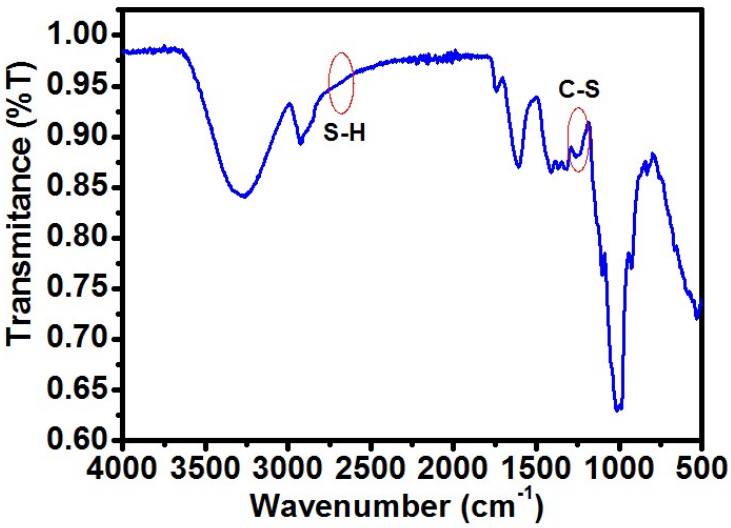

Fig. S1 FTIR spectra of onion biomass (source of OCDs)

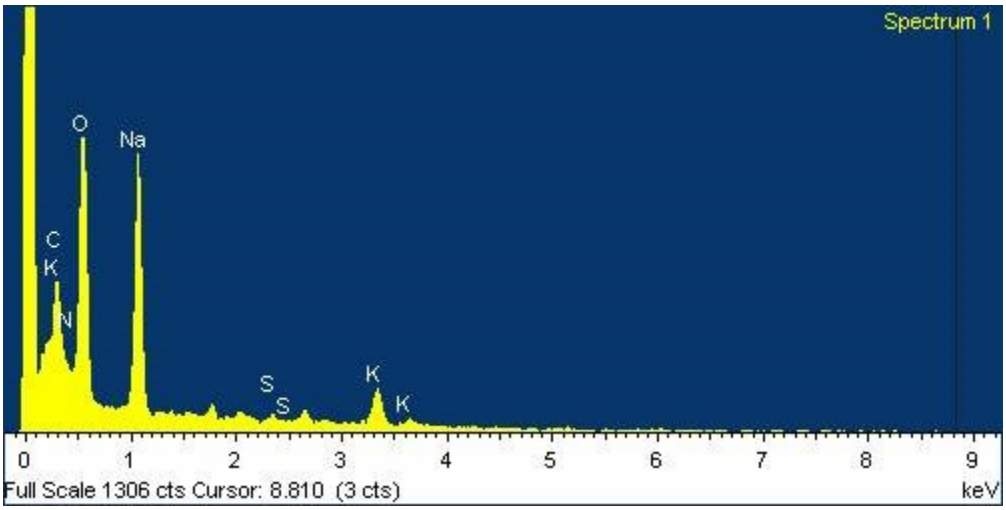

Fig S2: EDX spectrum of OCDs

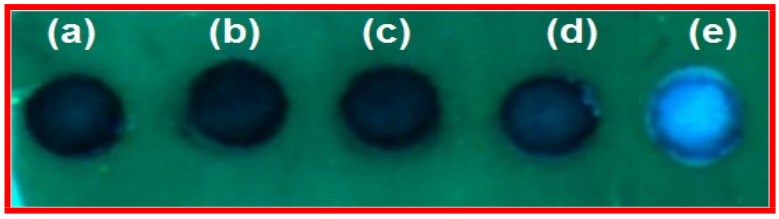

Fig. S3 Photographic images of sensor circles with (a) PNPs, (b) PNPs-$^{PSA}Ab_{cap}$, (c) PNPs-$^{PSA}Ab_{cap}$-PSA, (d) PNPs-$^{PSA}Ab_{cap}$-PSA-$^{PSA}Ab_{HRP}$ and (e) PNPs-$^{PSA}Ab_{cap}$-PSA-OCDs@$^{PSA}Ab_{HRP}$ under UV light with excitation wavelength at 365 nm.

Table S1. Comparison table with analytical performance of various optical biosensors reported in literature for detection of PSA

S. No.	Method Type	Linear range	LOD	References
1	Chemiluminescence resonance energy transfer (CRET)	1.0-100 ng/ml	600pg/ml	[5]
2	Colorimetric aptamers	1.0-100 ng/ml	20.0pg/ml	[6]
3	Fluorimetric aptamer-based assay	0.05-150 pg/ml	43fg/ml	[7]
4	Optical PL biosensor-based Zn-MOFs-NP	0.1-0.2 fg/ml	0.145fg/ml	[8]
5	Optical Biosensor	1-50 ng/ml	0.5 ng/ml	[9]
6	Optical Biosensor	1.0-250 ng/ml	0.72 ng/ml	[10]
7	Fluorescence	5ng/ml-120ng/ml	55.1 pg/ml	This work

Table S2: Analytical properties of GCE-PNPs-OCDs@HRP vs [H_2O_2]

S. No.	Properties	0.1 mg/mL HRP	0.5 mg/mL HRP	1 mg/mL HRP
1	Linear range (mM)	0-3	0-4	0-5
2	Sensitivity ($\mu A/mM/cm^2$)	128.8	136.3	154.3
3	LOD (mM)	0.0172	0.0068	0.0049

Optimization of experimental conditions

Optimization of experimental conditions like HQ and H_2O_2 concentration is important for conducting electrochemical detection. **Fig S4** shows the CV of $^{PSA}Ab_{cap}$ immobilized PANI NPs modified screen printed carbon electrodes (SPE) in PBS containing different concentrations of HQ in which a distinct redox peak was observed with 3 mM HQ. To optimize, H_2O_2 concentration, OCDs@$^{PSA}Ab_{HRP}$ was directly immobilized on PANI NPs via glutaraldehyde coupling over SPCE and cyclic voltammetry was conducted with sequential addition of H_2O_2 in presence of 3 mM HQ. At 4.5 mM of H_2O_2 maximum current was achieved and saturation was observed after further addition of H_2O_2. Hence 3 mM of HQ and 4.5 mM of H_2O_2 was utilized for all electrochemical experiments. Incubation time of capture antibody immobilization, PSA binding and probe binding was 30 min each during sensor construction.

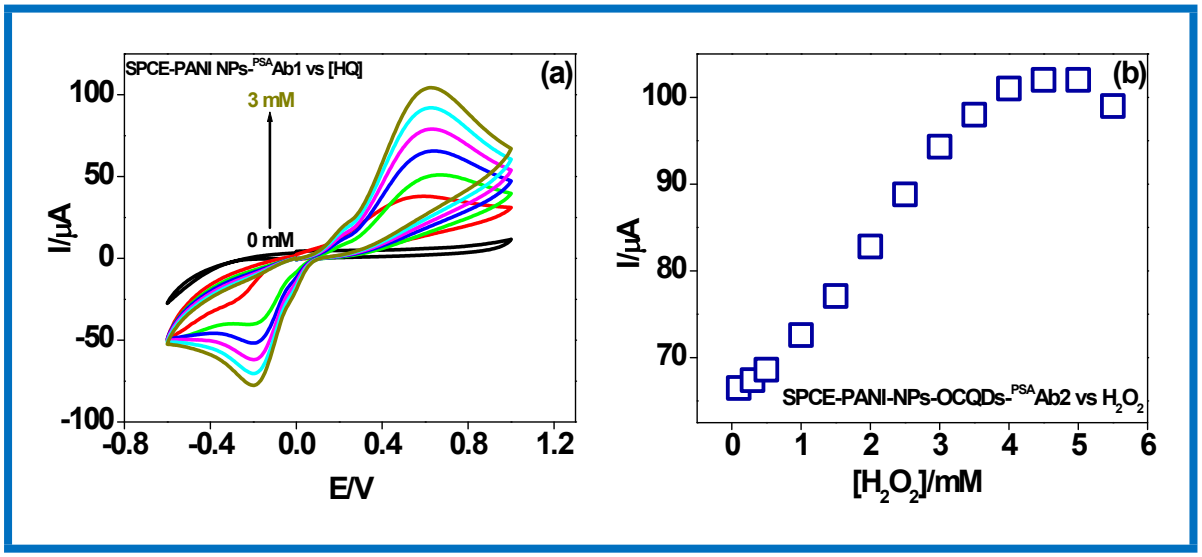

Fig S4: CV of PNPs modified SPE in PBS with different concentration of HQ at 50 mV/s and (b) CV response of OCDs@$^{PSA}Ab_{HRP}$ modified SPE-PANI NPs electrode with different concentrations of H_2O_2 in presence of PBS containing 3 mM HQ at 50 mV/s

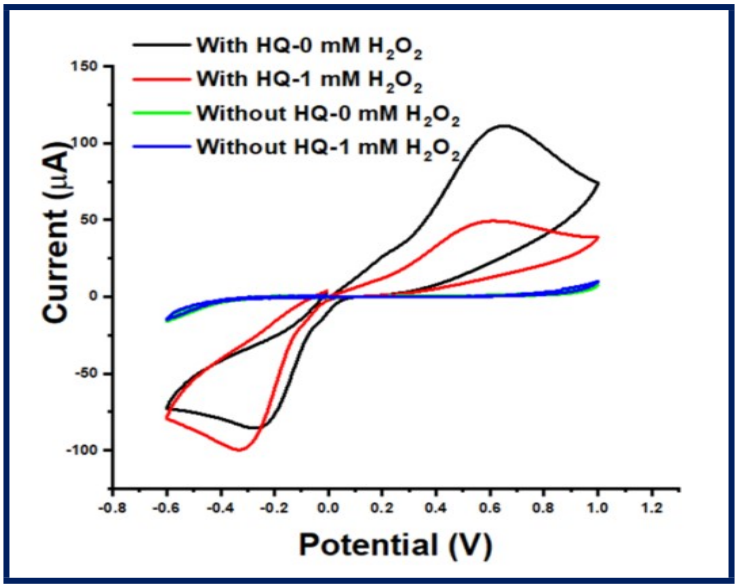

Fig S5: CV response of GCE-PNPs-OCDs@HRP in PBS with(blue) and without H_2O_2(green); CV response of GCE-PNPs-OCDs@HRP in PBS containing HQ with (black) and without H_2O_2(red).

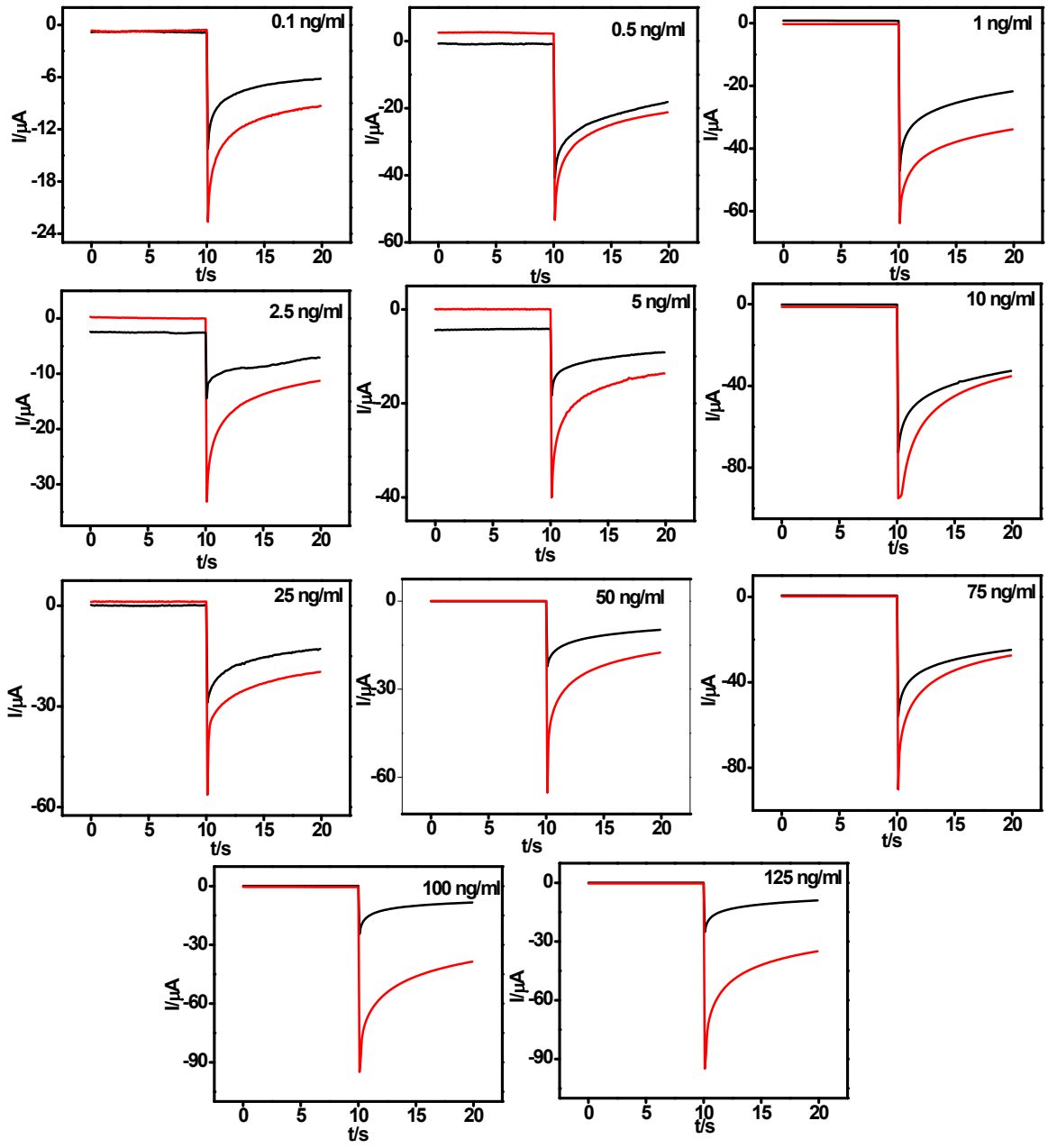

Fig S6: Amperometric curves of constructed biosensor at different concentration of PSA in PBS containing 3 mM HQ and 4.5 mM H_2O_2 (black curve: with HQ in absence of H_2O_2 and red curve: with HQ in presence of H_2O_2)

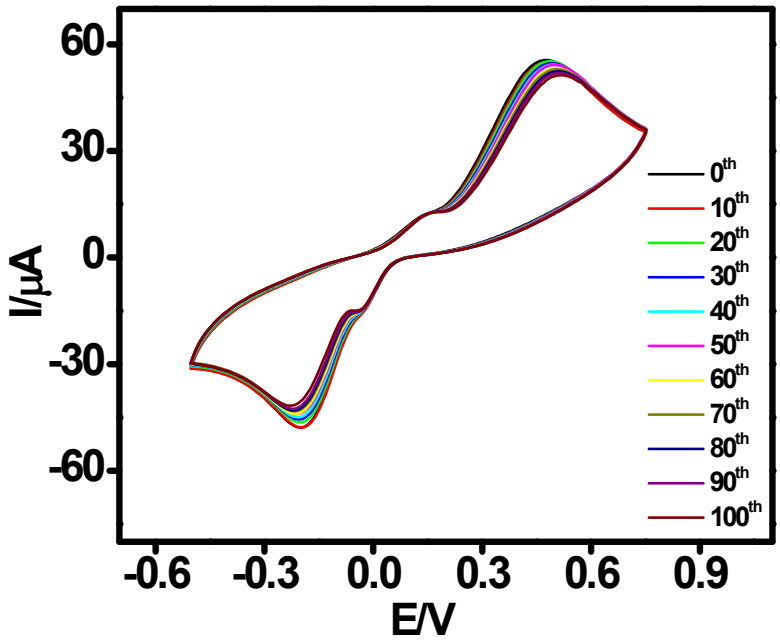

Fig S7: CV curves of 100 cycles of $^{PSA}Ab_{cap}$ modified sensor electrode in a PBS solution containing 3 mM HQ

Table S3: Comparison of analytical performance of electrochemical immunosensors reported in literature for detection of PSA

S. No.	Matrix	Probe	Linear range	LOD	References
1	rGO-silk peptide	hexacyanoferrate redox system	0.1-80 ng/ml	53 pg/ml	[11]
2	Chit-CNT	Methylene blue	0.85–12.5 ng/ml	0.75 ng/ml	[12]
3	Gold nanospears	Methylene blue	0.125–200 ng/ml	50 pg/ml	[13]
4	Gold nanospindles	Methylene blue	0.125 to 128 ng/ml	50 pg/ml	[14]
5	GO/Fe3O4 NSs	Ag/CdO NPs	50 pg/ml to 50 ng/ml	28 pg/ml	[15]
6	ferrocene (Fc)-labeled hairpin DNA	methylene blue (MB)-labeled DNA1-antibody	0.01 to 200 ng/ml	4.3 pg/ml	[16]
7	96-well plate-Avidin-BSA	silver enhancer solution	0.1 to 100 ng/ml	27 pg/ml	[17]
8	Au NPs	Au/Pt-polymethylene blue (PMB)	10 fg/ml to 100 ng/ml	2.3 fg/ml	[18]
9	Aptamer, mercaptophenylboronic acid	AgNPs	0.5–200 pg/ml	0.2 pg/ml	[19]
10	Au-rGO/Au-PMB/GCE	polydopamine-Au-horse radish peroxidase	1.0 fg/ml to 100 ng/ml	0.11 fg/ml	[20]
11	PNPs	OCDs@$^{PSA}Ab_{HRP}$	0.1-100 ng/ml	38 pg/ml	*Present work*

Table S4: PSA concentration in human serum sample determined by FLIA and ECIA and conventional CLIA

[PSA] in serum detected by proposed sensor (ng/ml)	[PSA] detected in serum by CLIA (ng/ml)	RSD (%)
0.75[#]	0.67	7.9
0.7[##]	0.67	3.1

Detected by [#]FLIA and [##]ECIA

Table S5: Spike-recovery of PSA in human blood serum sample detected by ECIA

[PSA] in serum by CLIA (ng/ml)	[PSA] spiked (ng/ml)	Total [PSA] detected (ng/ml)[#]	Recovery of PSA (%)
0.67	0	0.7	104.4
0.67	5	5.7	100.5
0.67	10	10.61	99.4

Table S6: Spike-recovery of PSA in human blood serum sample detected by FLIA

[PSA] in serum by CLIA (ng/ml)	[PSA] spiked (ng/ml)	Total [PSA] detected (ng/ml)[##]	Recovery of PSA (%)
0.67	0	0.75	111
0.67	5	5.85	103.1
0.67	10	10.6	99.3

References

1. D. G. Dastidar, P. Mukherjee, D. Ghosh and D. Banerjee, *Colloids Surf. A Physicochem. Eng. Asp.* 2021, **611**, 125781.
2. K. Bankoti, A. P. Rameshbabu, S. Datta, B. Das, A. Mitra and S. Dhara*, J. Mater. Chem. B,* 2017, **5**, 6579-6592
3. S. Zhao, M. Lan, X. Zhu, H. Xue, T. W. Ng, X. Meng, C. S. Lee, P. Wang and W. Zhang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 17054–17060.
4. J. Ren, L. Stagi and P. Innocenzi, *Prog. Solid. State Chem*., 2021, **62,** 100295.
5. J. Chong, H. Chong and J.H. Lee*, Anal. Biochem*., 2019,**564**,102-107
6. O.H. Shayesteh and R. Ghavami*, Spectrochim. Acta part A Mol. Biomol. Spectrosc.* 2020, **226**, 117644.
7. M. Chen, Z . Tang, C. Ma and Y. Yan, *Sens. Actuators B chem.* 2020,**302**, 127178.
8. S.M. El-Sheikh, S.M. Sheta, S.R. Salem, M.M. Abd-Elzaher, A.S. Basaleh and A.A . Labib, *Biosensors*, 2022 ,**12**,931.
9. R.M. Kong, X .Zhang, L. Ding, D. Yang and F. Qu*, Anal. Bioanal. Chem.,* 2017, **409**, 5757–5765.
10. F. Duan, S. Zhang, L. Yang, Z. Zhang, L. He and M. Wang, *Anal. Chim. Acta* 2018, **1036**, 121–132.
11. Y. Wang, Y. Qu, G. Lui, X. Hou, Y. Huang, W. Wu, K. Wu, and C. Li, *Microchim. Acta.*, 2015, **11**, 2061.
12. F. Tahmasebi and A. Noorbakhsh, Electroanalysis, 2016, **28**, 1134.
13. A. Rahi, N. Sattarahmady, and H. Heli, Talanta, 2016, **156**, 218.
14. N. Sattarahmady, A. Rahi, and H. Heli, Sci. Rep., 2017, **7**, 11238.
15. Y. Zhao, L. Cui, Y. Sun, F. Zheng, and W. Ke, *ACS App. Mater. Interfaces*, 2018, **11**, 3474.
16. Ren, K., Wu, J., Yan, F., Zhang, Y., & Ju, H. *Biosens. Bioelectron*, 2015, **66**, 345-349.
17. A. Parnsubsakul, R. E. Safitri, P. Rijiravanich, and W. Surareungchai, *J. Electroanal. Chem.*, 2017, **785**, 125.
18. J. Zhao and Z. Ma, *Biosens. Bioelectron.,* 2018, **102**, 316.

19. N. Xia, C. Cheng, L. Liu, P. Peng, C. Liu, J. Chen. *Microchim. Acta.* 2017, **184**, 4393–4400 ().

20. Z. Tang, L. Wang, and Z. Ma, *Biosens. Bioelectron.,* 2017, **92,** 577.